Casein Denaturation through Probiotic Microorganism Producing Enzyme Dipeptidyl Peptidase4

A synopsis of the proposed thesis to be submitted for the degree of

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In

DAIRY TECHNOLOGY (MICROBIOLOGY)

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INTRODUCTION

Milk is the primary source of nutrition for infant mammals. It contains nutrients like energy, water, carbohydrate, fat, protein, vitamins, minerals, and minor Biological proteins &enzymes (lactoferrin, lacto peroxidase, lipases) . People therefore converted milk to curd, cheese and other products to reduce the levels of lactose. Thousands of years ago, a chance mutation spread in human populations in Europe that enabled the production of lactase in adulthood. This mutation allowed milk to be used as a new source of nutrition which could sustain populations when other food sources failed. People process milk into a variety of products such as cream, butter, yogurt, kefir, ice cream, and cheese. Modern industrial processes use milk to produce casein, whey protein, lactose, condensed milk, powdered milk, and many other food-additives and industrial products. (Pehrsson et al, 2003)

There is also a problem with digestion of milk which is termed as lactose intolerance. Approximately 70% of population worldwide belonging to different races and regions have this problem of intolerance, also known as "Dairy intolerance."(Lomer *et al*, 2008)

DAIRY INTOLERANCE vs TRUE ALLERGY vs LACTOSE INTOLERANCE

Dairy intolerance can present in a wide array of annoying symptoms. Dairy intolerance should not be confused with a true allergy or milk allergy to dairy products. For most people who have dairy intolerance, it is simply unpleasant to consume dairy foods that trigger the annoying symptoms, but for those with true allergies, it may trigger a serious health problem and potentially even be life threatening. Some typical examples of dairy intolerance are digestive problems (such as nausea, stomach pain, gas, bloating and bowel disturbances) and respiratory problems (such as congestion of the sinuses or chest, headaches from sinus congestion and sorethroat). (Vandenplas, *2015)*

Most dairy intolerance is caused from foods that still contain significant amounts of the milk solids. The milk solids in dairy products are where the proteins and sugars are found. Casein, the predominate proteins found in milk and lactose, one of the sugars, are two of the biggest causative agents for dairy intolerance. In general, the liquid portion of milk (the whey and butterfat) is not what causes dairy intolerance (or allergic reactions) but if they do, trace remnants of milk solids that remain

in with the whey or butterfat are the likely culprits for triggering problems. Commercial cow milk is a common problem for those who are intolerant to lactose. It has a significant amount of milk proteins and sugars. Many people who have dairy intolerance simply avoid consuming dairy products. This is unfortunate, because there are so many important nutrients found in dairy and there are sometimes simple solutions to manage dairy intolerance.

LACTOSE INTOLERANCE

Lactose intolerance is probably the most common dairy intolerance problem- the inability to break down lactose, so that your body can assimilate it. Lactase is the enzyme that digests the milk sugar lactose. If lactose remains in a dairy product you need to have this enzyme present to metabolize this sugar. Lactase can be produced by certain cells that line your digestive tract. However, many people are unable to produce lactase. This may be genetic (you do not have the needed genes to produce this enzyme) or it may be an acquired defect (something has damaged your digestive tract, preventing it from producing lactase). Approximately 70% of the population worldwide belonging to different regions and areas were lactose intolerant Consuming real, unprocessed milk or raw milk cheeses from pasture-fed cows can solve this problem for many. That's because these dairy have live, beneficial lacto-bacillus bacteria present. These "good" bacteria actually help you break down the lactose. When the dairy product is pasteurized, as it is with most commercial milk and cheeses, these beneficial bacteria are unfortunately destroyed. However, dairy products from animals grazing on organic pastures are the "gold standard" in obtaining excellence when you are looking at the health benefits and nutrient value of the products. These types of real, whole milk dairy products offer a complete source of protein, vitamins and minerals, and lots of pro-biotic, beneficial bacteria which produce helpful enzymes (such as lactase) and vitamins (such as folic acid, niacin, riboflavin, B6 & B12) that your body needs to function optimally.(Deng *et al*,2015)

TRUE ALLERGY

A milk allergy is an immune reaction to one of the many proteins in animal milk. It's most often caused by the alpha S1-casein protein in cow's milk. A milk allergy is sometimes confused with

lactose intolerance because they often share symptoms. The two conditions are very different, however. Lactose intolerances occurs when a person lacks the enzyme (lactase) to metabolize lactose — a milk sugar — in the intestines (Ross *et al*, 2013) .Then you need to choose products that have had most of the milk solids removed from them. As mentioned earlier, the casein milk proteins are the most problematic dairy proteins which trigger both dairy intolerance and true allergic reactions. It has also been found to worsen the symptoms of autistic children. (Casein strongly affects the symptoms of autistic children, along with gluten, another protein found in many grains.). Some people are not able to digest lactose, the sugar in milk, after they are weaned, because they do not produce enough of an enzyme known as lactase. Lactase is needed to digest milk properly. As concerns about lactose intolerance and milk allergies widen, a range of substitute milks, such as almond and soy milk, have become available. In thinking of digestion we can use some probiotic bacteria's which can produce enzymes helpful of protein denaturation, (Silanikove*et al*, 2015). So we are using a probiotic bacteria and an Enzyme for denaturation of casein, which helps in digestion of every aged person. The enzyme which I Am going to use is" Dipeptidyl peptidase4'' through a probiotic bacteria" Lactobacillus Helveticus".

Probiotics are living bacteria and yeasts that provide health benefits when consumed in large amounts.They can be taken as supplements or consumed naturally through fermented foods like yogurt, kefir, sauerkraut, kimchi and kombucha. Lower risk of infection was documented whereas improved digestion and health benefits were revealed more. (Saarela et al, 2000) .

Dipeptidyl Peptidase4: Dipeptidyl Peptidase4 is the enzyme produced by probiotic bacteria "Lactobacillus Helveticus". Dipeptidyl peptidase-4 (DPP4), also known as adenosine deaminase complexing protein 2or CD26 (cluster of differentiation 26) is a protein that, in humans, is encoded by the *DPP4* gene.DPP4 is related to attractin, FAP (Fibroblast Activation Protein), DPP (Dipeptidyl Peptidase) The protein encoded by the *DPP4* gene is an antigenic enzyme expressed on the surface of most cell types and is associated with immune regulation, signal transduction and apoptosis. It is an intrinsic membrane glycoprotein and a serineexopeptidase that cleaves X-proline dipeptides from the N-terminus of polypeptides8 and DPP9. (Kameoka *et al*, *2006).*

LACTOBACILLUS HELVETICUS

Lactobacillus helveticus is a lactic-acid producing, rod-shaped bacterium of the genus *Lactobacillus*. The bacterium's specific name is an adjective derived from "Helvetia", the Latin name for the region occupied by the ancient Helvetii (and for modern Switzerland). (Van et al, 2008) It is most commonly used in the production of American Swiss cheese. It is also a probiotic bacteria, the primary function of *L. helveticus* culture is to prevent bitterness and produce nutty flavors in the final cheese. (Aihara*et al, 2005).* The primary function of *L.helveticus*culture is to prevent bitterness and produce nutty flavors in the final cheese. In Emmental cheese production, *L. helveticus*is used in conjunction with a *Propionibacterium*culture, which is responsible for developing the holes (known as "eyes") through production of carbon dioxide gas. Ingestion of powdered milk fermented with *L. helveticus* was shown to decrease blood pressure due to the presence of manufactured tripeptides that have ACE inhibitor activity.Aside from the gut, 'friendly' bacteria like *Lactobacillus helveticus* can be traced back to certain external sources that consequently restore the gut's average CFUs (colony-forming units).A normal individual's gastrointestinal tract usually contains between 1-100 billion CFUs of good' and 'bad' bacteria alike. For optimal digestive and immune functionality, you need to rely on a 4:1 ratio in favor of probiotics. A reversed pattern of this delicate balance could result in unpleasant side effects like gas, bloating, visceral pain, and a considerably weakened immune system. Lactobacillus helveticus plays a particularly important role in this sense, since it allows for improved lactose tolerance; meliorated bone wellness, and normalized cardiovascular health. Hence, *L.helveticus* was first isolated at the beginning of the 20th century and actually considered to be a part of the *Bacillus* genus.Its taxonomic evolution from that point onward remains therefore marked by numerous misidentifications and false categorizations that have placed it under different names and properties as well. *L.helveticus* can be added to soft cheeses (namely types of cheese obtained without the involvement of heat) so as to soften the bitterness otherwise associated with an advanced fermentation activity and transform it into a nuttier aroma. (Simone Guglielmetti, 2012)

Examples of cheese making techniques involving *Lactobacillus helveticus* can be found in the case of Mozzarella, Cheddar, Parmesan, Romano, etc.

LACTOBACILLUS DELBRUEKII Sub sp BULGARICUS (LB87)

Lactobacillus delbrueckii* subsp. *bulgaricus (until 2014 known as ***Lactobacillus bulgaricus***) is the main bacteria used for the production of yogurt. It is also found in other naturally fermented products. First identified in 1905 by the Bulgarian doctor Stamen Grigorov, the bacterium feeds on lactose to produce lactic acid, which is used to preserve milk. *Lactobacillus delbrueckii* subsp. *bulgaricus* is commonly used alongside *streptococcus thermophilus* as a starter for making yogurt. The two species work in synergy, with *L. d. bulgaricus* producing amino acids from milk proteins, which are then used by *S. thermophilus*. Both species produce lactic acid, which gives yogurt its tart flavor and acts as a preservative. The resulting decrease in pH also partially coagulates the milk proteins, such as casein, resulting in yogurt's thickness. While fermenting milk, *L. d. bulgaricus* produces acetaldehyde, one of the main yogurt aroma components. Some strains of *L. d. bulgaricus*, such as *L. bulgaricus* GLB44, also produce bacteriocins, which have been shown to kill undesired bacteria *in vitro*.

Some of the biggest importers of the bacterium are Japan, the United States, and the European Union.

It has also been considered a contaminant of beer due to its homo fermentative production of lactic acid, an off-flavor in many styles of beer. In other styles of beer, however, lactic acid bacteria can contribute to the overall appearance, aroma, taste, and/or mouth feel, and generally produce an otherwise pleasing sourness.(Courtin,2003).

Yoghurt has long history of recognition as a dietary product with many desirable effects. It is made from the symbiotic growth of S. thermopiles and Lb. delbrueckii subsp. bulgaricus. These yoghurt starter bacteria do not survive the gastric passage or colonies the gut and consequently do not play a role in the human gut. Hence, the recent trend is to add Lb. acidophilus, Bifidobacterium spp. and *Lb. casei* to yoghurt. Modzelewska-Kapituła et al. (2008) investigated the possibility of using two probiotic strains of *Lb. plantarum* and *Lb. fermentum* in yoghurt production. These strains did not acidify milk during 24 h and 72 h fermentation at 37 °C, but grew well and remained at the level of 108 cfu/mL during 21 days of cold storage. The authors proposed their application to yoghurt production along with commercial starter culture viz., *Lb. delbrueckii subsp. bulgaricus* and *S. thermophilus* in obtaining products with typical sensory properties, pH values and numbers of

potentially probiotic bacteria at desired level of 107 cfu/mL With the growing popularity of yoghurt as probiotic carrier food, yoghurt is emerging as a new therapeutic leading edge with different diseases. A number of health benefits have been claimed for probiotic strains.(Modzelewska,2008)

Dietary supplementation with yoghurt is considered as a simple, effective, and safe treatment that decreases the incidence and duration of antibiotic associated diarrhoea (Beniwal et al., 2003). Yoghurt consumption is also useful to alleviate inflammatory bowel disease (IBD), which involves two major disorders such as ulcerative colitis and Crohn's disease. The way by which yoghurt exerts such effect is suggested to be through the immune mechanisms, with an increased in IgA+cells, capable of modulating the inflammatory response with a diminution in CD4+ and CD8+ population and an increase in the apoptosis of the infiltrative immune cells.

REVIEW OF LITERATURE

Casein is a phosphoprotein comprising c. 80% of the proteins in cow's milk and between 20% and 45% of the proteins in human milk. Denaturation of casein is done to disrupt the normal alpha-helix and beta sheets in a protein and uncoils it into a random shape. By using probiotic organisms producing Dipeptidyl peptidase4 enzyme will cleave the casein protein and help for better digestion in casein intolerance people. On the basis of this the review has been tabulated.

PAPER	WORK	AUTHORS AND	YEAR	JOURNAL
1.Probiotic bacteria : safety, functional and technological properties.	Studied that the s probiotic (health promoting) micro-organisms have been increasingly included in various types of food products, especially in fermented milks. By taking Several aspects, including safety, functional and technological characteristics, into consideration in the selection process of probiotic micro-organisms. Safety aspects include specifications such as origin (healthy human GI-tract), non-pathogenicity and antibiotic resistance characteristics. Functional and technological properties, can be used to identify the effects for GI-infections and allergies.	Saarela et al	2000	Journal of biotechnology

2. Effect of milk supplementation and culture composition on acidification, textural properties and microbiological stability of fermented milks containing probiotic bacteria	Studied that Milk supplementation and culture composition had significant effects on acidification, texture, and microbiological stability of fermented milks containing probiotic bacteria. addition of Casein hydrolysate (CH) and use of mixed cultures resulted in a better acidification and enhanced textural properties. Probiotic cell count achieved was more than 2.2 107 CFU mL1 in fermented milk after 7 days of storage at 41C.	Oliveira et al	2001	International Dairy Journal
3. Effect of pH on the Association of Denatured Whey Proteins with Casein Micelles in Heated Reconstituted Skim Milk.	Studied and explained that Skim milk was adjusted to pH values between 6.5 and 6.7 and heated (80, 90, and 100 °C) for up to 60 min. Changes in casein micelle size, level of whey protein denaturation, and level of whey protein association with the micelles were monitored for each milk sample. Changes in casein micelle size were markedly affected by the pH at heating. changes in casein micelle size induced by the heat treatment of skim milk were a consequence of the whey proteins associating with the casein micelles and that the level of association was markedly influenced by small pH changes of the milk.	Skelte et al	2003	Journal of agriculture and food chemistry
4. Influence of Residual Milk-Clotting Enzyme on αs1 Casein Hydrolysis During Ripening of Reggianito Argentino Cheese	The aim of the work is to investigate the influence of residual milk-clotting enzyme on α-casein hydrolysis in Reggianito cheese with different cooking temperatures (45,52,60). Milk-clotting enzyme residual activity in cheeses was assessed using a chromatographic method, and the hydrolysis of αs1-casein was determined by electrophoresis and high performance liquid chromatography	Hynes et al	2004	Journal of Dairy science
5. Surface-layer protein extracts from Lactobacillus Helveticus inhibit enterohaemorrhagic Escherichia	These in vitro studies indicate that a non- viablr constituent derived from a probiotic strain may prove effective in interrupting the infectious process of an intestinal pathogen through inhibition of pathogen adherence.	Henry et al	2006	Cellular microbiology

coli157:H7 adhesion to epithelial cells				
6. Putting microbes to work: Dairyfermentation, cell factories and bioactive peptides. Part II: Bioactive peptide functions	Fermentation of milk proteins using the proteolytic systems of lactic acid bacteria is an attractive approach for generation of functional foods enriched in bioactive peptides given the low cost and positive nutritional image associated with fermented milk drinks and yoghurt.	Stanton et al	2007	Journal of biotechnology
7. Effect of pH on the Association of Denatured Whey Proteins with Casein Micelles in Heated Reconstituted Skim Milk	This study indicate that the changes in casein micelle size induced by the heat treatment of skim milk were a consequence of the whey proteins associating with the casein micelles and that the level of association was markedly influenced by small pH changes of the milk.	Skelete et al	2003	Journal of agriculture and food chemistry
8.Food processing increases casein resistance to simulated infant digestion.	The objective of this study was to determine whether processing could modify the resistance of casein (CN) to digestion in infants.	Dupont et al	2010	Molecular nutrition food research
9. Effect of Powdered Fermented Milk with Lactobacillus helveticus on Subjects with High-Normal Blood Pressure or Mild Hypertension	Studied that Two tripeptides (Val-Pro-Pro and Ile-Pro-Pro) that have inhibitory activities for angiotensin I-converting enzyme are produced in milk fermented with Lactobacillus helveticus. In this study we evaluated the effect and safety of powdered fermented milk with L. helveticus CM4 on subjects with high-normal blood pressure or mild hypertension	Aihara et al	2005	Journal of the American college nutrition
10. The effect of Lactobacillus helveticus fermented milk on sleep and health perception in elderly subjects	This study included that two intervention periods of 3 weeks each, separated by a 3-week washout period. Subjects took 100 g of fermented milk drink or a placebo drink (artificially acidified milk) daily in the first supplementary period and the other drink in the second supplementary period. For each period, we measured sleep quality by means of actigraphy and a sleep questionnaire, and assessed the quality of life (QOL) by SF-	Yamamura et al	2007	Europian journal of clinical nutrition

	36 health survey			
11.Characterization of immune-active peptides obtained from milk fermented by Lactobacillus helveticus	• The objectives of this research were to confirm the effect of compounds derived from milk fermented by Lactobacillus helveticus (LH-2) on the nonspecific host defence system, and isolate and characterize the active peptides that mediate the immune response.	Tellez et al	2010	Journal research of dairy
12. Streptococcus thermophilus and Lactobacillus delbrueckii subsp. bulgaricus survive gastrointestinal transit of healthy volunteers consuming yogurt	• The present study indicates that substantial numbers of yogurt bacteria can survive human gastrointestinal transit.	Mater et al	2005	FEMS microbiology letters
13. Disruption of Lactobacillus delbrueckii ssp. bulgaricus 11842 cells for lactose hydrolysis in dairy products: a comparison of sonication, high-pressure homogenization and bead milling	• The objectives of this research were to study the Cultures of Lactobacillus delbrueckii ssp. bulgaricus 11842 grown on whey supplemented with yeast extract were subjected to treatments using sonication, a high-speed bead mill, and a high-pressure homogenizer. The various means of disruption were compared by the release of intracellular β -galactosidase and by scanning electron microscopy SEM. The activity of β-galactosidase after homogenization is observed.	Bury et al	2001	Innovative food science and emerging technologies
14. Survival of Lactobacillus delbrueckii ssp. bulgarlcus Following Spray-Drying	• This study indicated that Survival of cell concentrates of Lactobacillus delbrueckii ssp. bulgaricus following spray-drying in skim milk with and without ascorbic acid and monosodium glutamate was evaluated during storage at different conditions of temperature and water activity. High temperature and high water activities were detrimental to survival of dried cells.	Teixeira et al	1995	Journal of Dairy science
15. Biofunctional properties of	• The study indicates that Milk protein derived bioactive peptides are shown to have	Patil et al	2015	Asian journal of Dairy and food Research.

milk protein derived bioactive peptides.	antihypertensive, antimicrobial, immunomodulatory, antioxidative and mineral-binding properties. Bioactive peptides derived from milk proteins are of particular interest to the food industry due to the potential functional and physiological roles that they exhibit.			
16. Effects of long-term intake of a yogurt fermented with *Lactobacillus delbrueckii* subsp. *bulgaricus* 2038 and *Streptococcus thermophilus* 1131 on mice.	• The study indicates that long-term intake of LB81 yogurt has the potential to maintain systemic homeostasis, such as the gut barrier function, by controlling the intestinal microbiome and its metabolites.	Yuki et al	2018	International Immunology

OBJECTIVE

1. Isolation and Identification of enzyme secreted probiotic microbe from yogurt.
2. Effect of Enzyme and probiotic microorganism on milk.
3. Chemical Analysis of the final Formation of probiotic casein.(final product).

METHODLOGY

1. Isolation of probiotic microorganism: Isolation of the probiotic microorganism is done from yogurt sample through the process of pure culture techniques. Using MRS or M17 media. The Fermented milk sample is added to 9.9ml sterile tryptone dilluent. Appropriate dilutions were made and subsequently pour-plated in to duplicate onto selective media. (Oliveira et al, 2001). One gram of each sample will be dissolved in 100ml of MRS broth initially then plating will be carried out. (Hoque et al, 2010).

2. Identification and confirmation of microorganism: Identification and confirmation is done by performing Biochemical tests. Growth at 8 and 15 °C in tubes containing MRS broth, growth in 7.5% NaCl, and fermentation of carbohydrates and other chemical tests were determined.

(Sneath et al, 1986)

3. Inoculating probiotic microorganism: Inoculation of bacteria in milk is done after isolating the organism from the yogurt sample. (Oliveira et al, 2001)

4. **Effect of enzyme on milk:** The enzyme action is checked by performing different tests like

a. SDS-Phage
b. ELISA
c. Immunoblotting

(a) SDS-Phage: A very common method for separating proteins by electrophoresis uses a discontinuous polyacrylamide gel as a support medium and sodium dodecyl sulfate (**SDS**) to denature the proteins. The method is called sodium dodecyl sulfate polyacrylamide gel electrophoresis (**SDS-PAGE**). (Skelete et al, 2003)

(b) ELISA test: The enzyme- linked immunosorbent assay (ELISA) is a test that uses antibodies and color change to identify a substance. ELISA is a popular format of "wet-lab" type analytic biochemistry assay that uses a solid-phase enzyme immunoassay (EIA) to detect the presence of a substance. The main principle behind ELISA is Basis and Extension. Enzyme-linked Immunosorbent Assays (ELISAs) combine the specificity of antibodies with the sensitivity of simple enzyme assays, by using antibodies or antigens coupled to an easily-assayed enzyme. ELISAs can provide a useful measurement of antigen or antibody concentration.(DuPont et al,2010)

(C) Immunoblotting: The western blot (sometimes called the protein immunoblot) is a widely used analytical technique used in molecular biology, immunogenetics and other molecular biology disciplines to detect specific proteins in a sample of tissue homogenate or extract.(DuPont et al,2010). Enzyme secreted by the probiotic microorganisms will be estimated through Enzyme assays. (Shihata et al, 2000)

5. Identification of casein before and after denaturation: Denaturation of proteins involves the disruption and possible destruction of both the secondary and tertiary structures. Since denaturation reactions are not strong enough to break the peptide bonds, the primary structure (sequence of amino acids) remains the same after a denaturation process. Denaturation disrupts the normal alpha-helix and beta sheets in a protein and uncoils it into a random shape. Denaturation occurs because the bonding

interactions responsible for the secondary structure (hydrogen bonds to amides) and tertiary structure are disrupted. In tertiary structure there are four types of bonding interactions between "side chains" including: hydrogen bonding, salt bridges, disulfide bonds, and non-polar hydrophobic interactions. Which may be disrupted Therefore, a variety of reagents and conditions can cause denaturation. The most common observation in the denaturation process is the precipitation or coagulation of the protein. (DuPont et al, 2010)

6. Checking the properties of final product: The final product properties are checked for its nutritional and probiotic properties. Several aspects like safety, functional and technological characteristics have to be taken in to consideration during final product analysis. (Saarela et al, 2000)

FLOW CHART OF PROCESS:

Isolation of microorganism

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Identification and confirmation of probiotic bacteria

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Inoculation of probiotic bacteria in to milk

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Identification of amount of enzyme production after inoculation of probiotic bacteria

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Identification of casein before inoculation of bacteria

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Denaturation done at different temperatures

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Identification of casein after inoculation in milk

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Final obtained denatured product

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Checking the properties of final product

SIGNIFICANCE

Significance of Dipeptidyl Peptidase4

Dipeptidyl peptidase-4 (DPP4) or Adenosine Deaminase Complexing Protein 2 (ADCP 2) orT-cell activation antigen CD26is aserine exopeptidase belonging to the S9B protein family that cleaves X-proline dipeptides from the N-terminus of polypeptides such as chemokines, neurokines and peptidehormones. The protein encoded by the DPP4 gene is an antigenic enzyme expressed on the surface of most cell types and is associated with immune regulation, signal transduction and apoptosis. It is an intrinsic membrane glycoprotein and a serine exopeptidase that cleaves X-proline dipeptides from the N-terminus of polypeptides. It is a rather indiscriminate enzyme for which a diverse range of substrates are known. The substrates of CD26/DPPIV are proline (or alanine)-containing peptides and include growth factors, chemokines, neuropeptides, and vasoactive peptides. DPP4 is related to attractin, FAP, DPP8 and DPP9. (Balbo, 2012).

CD26/DPPIV plays an important role in tumor biology, and is useful as a marker for various cancers, with its levels either on the cell surface or in the serum increased in some neoplasms and decreased in others. A new class of oral hypoglycemics called dipeptidyl peptidase-4 inhibitors work by inhibiting the action of this enzyme, thereby prolonging incretin effect in vivo. A new coronavirus related to SARS, named Middle East respiratory syndrome coronavirus, has been found to bind to DPP4. DPP4 is found on the surface of cells in the airways (such as the lungs) and kidneys. Scientists may be able to use this to their advantage by blocking the virus's entry into the cell. DPP4 plays a major role in glucose metabolism. It is responsible for the degradation of incretins such as GLP-1.

Dipeptidyl peptidase IV (DPP-IV) belongs to the exopeptidase class of proteolytic enzymes. Exopeptidases cleave the N- or C-terminal amino acid residues from peptides and proteins. Exopeptidases that cleave N-terminal residues are referred to as aminopeptidases, while those that cleave C-terminal residues are called carboxypeptidases. DPP-IV has the very uncommon capacity to cleave the peptide bond following a penultimate proline thereby releasing proline-containing dipeptides from the N-terminus of polypeptide chains. DPP-IV is technically referred to as a prolyl-amino-dipeptidase. It is one of the few enzymes able to facilitate the digestion of proline-rich proteins and polypeptides. The specific activity of the enzyme is, it is normally expressed by the cells of the

intestinal brush border and is essential for the complete breakdown of proline-containing dietary proteins is such as gluten and casein. . These allergenic proteins are highly resistant to hydrolysis by other proteolytic enzymes and have been implicated in a number of serious adverse food reactions including the enteropathic manifestations of celiac disease. All of the toxic and immunogenic peptide sequences of gliadin contain proline. DPP-IV activity contain enzymes with a broad spectrum of exo- and endopeptidase activities for which HUT(Hemoglobin Unit on the Tyrosine) units are an appropriate measure of proteolytic ability basis. Hemoglobin Unit on the Tyrosine (HUT) is a proteolytic enzyme unit used to express Dipeptidyl peptidase 4 activity on their products. (Diana Röhrborn july2015)

Significance of *Lactobacillus Helveticus*:

Aside from lactic acid, probiotics like *L.helveticus* can also prompt the creation of hydrogen peroxide, which greatly helps the human body in terms of immunity protection against all kinds of pathogens (whether airborne, ingested, etc.).This substance then inhibits viruses, microbes, and fungi from expanding into larger colonies in the long term and, as a result, shields the organism against health threatening illnesses. *L.helveticus* can hence be linked with immunomodulatory properties such as reduced inflammation, restored mucosal unity, and even antimutagenic effects in time. *Lactobacillus* probiotics naturally increase their hydrogen peroxide production when they encounter pathogen infections. From gastrointestinal and immune protection to cardiovascular, bone, and mental wellness, it would seem that *L.helveticus* can cover a wide array of 'good' points inside the human body. While scientific findings might still be at the beginning of their discoveries regarding this probiotic in particular, the results gathered so far appear to be more than promising for *L.helveticus* and its contribution to one's body and mind.(Giorgio Giraffa, 2014)

Lactobacillus helveticus to improved bone structure in terms of mineral content and density by suggesting that this 'good' bacterium enables for better calcium absorption, as well as for a more resistant skeletal structure (especially for postmenopausal women, who often have to face bone problems such as osteoporosis, for example).It can promote a beneficial action on your mental state too. Because of the brain-gut axis, your mind and gastrointestinal system are innately linked,

which means that they can mutually influence one another to a great extent at all times. Severe behavioral disturbances like depression and anxiety have been shown to become significantly more manageable when treated in connection with habitual *L.helveticus* enhancement. The promotion of gastrointestinal wellbeing is definitely one of the main reasons why *L.helveticus* has quickly become such a popular probiotic choice all around the world.

Cremonesi et al. (2013) mentioned the potential of *L. helveticus* to produce peptides with a biological function, such as those possessing an inhibitory activity on the angiotensin converting enzyme (ACE), demonstrating the therapeutic value of this species when used in fermented dairy products. These peptides have been shown in clinical trials (Jauhiainen et al., 2005). It has the potential to produce bioactive peptides such as angiotensin converting enzyme inhibitory activity in fermented dairy products, demonstrating the therapeutic value of this species. A most intriguing feature of the genome of *L. helveticus* DPC4571 is the remarkable similarity in gene content with many intestinal lactobacilli, although originating from considerably different environments. The effect of peptides released during the fermentation of milk on the humoral immune system and on fibro sarcoma growth was studied. *L.helveticus* was able to release peptidic compounds during milk fermentation due to its high proteolytic activity, as was shown by the degree of proteolysis and size-exclusion HPLC elution profiles (J.G. leblanc, 2002). *L.helveticus* produces angiotensin-converting enzyme (ACE)-inhibitory peptides that can prevent or control high blood pressure *L.helveticus* fermented milk has a BP-lowering effect in hypertensive subjects, Milk fermented with *L.helveticus* can enhance specific and non-specific immunity. *L.helveticus* has the ability to **inhibit different pathogenic microorganism**and has beneficial effects on the intestine, the vaginal mucosa, or the pharyngeal epithelium.It has antioxidant properties. Administration of *L.helveticus* to healthy human subjects resulted in a significant increase in butyrate beneficial for gut homeostasis. *L.helveticus* alone or in combination with *S. thermophiles* effectively **reduces the antigenicity** of α-lactalbumin and β-lactoglobulin, the **major allergens in cow's milk.it increased blood calcium level inelderlyvolunteers.** *L.helveticus* fermented milk whey contains bioactive components that **increasebone formation.** When healthy volunteers took *L.helveticus*, together

with *Bifidobacterium longum*, **psychological stress levels, including depression, were decreased**in subjects who took the probiotics regularly (Joseph Cohens, 2017)

***LACTOBACILLUS DELBRUEKII Sub sp BULGARICUS** (LB87)*

Lactobacillus delbrueckii is a rod shaped, lactic acid-producing, facultative anaerobic bacterium that is non-pathogenic, non-toxigenic, non-motile and non-spore forming. *L. delbrueckii subspecies bulgaricus* (herein after referred to as *L. bulgaricus*) has been used historically in the manufacture of dairy products, including yogurt and a number of cheeses. It is established as a widely-used starter culture in the dairy industry, and has traditionally been used together with *Streptococcus thermophilus* in the manufacturing of yogurt (Hao, 2011, Stamatova, 2007). The synergistic relationship between *L. bulgaricus* and *S. thermophilus* as thermophilic starter cultures has been demonstrated historically, and these two bacterial cultures have been recognized as the only required ingredients for yogurt production (ISO 7889:2003 |IDF 117).

It has also been demonstrated that genomic variation within strains of *L. bulgaricus* render differences in texture during yogurt production (Hao, 2011). *L. bulgaricus Lb-87* was isolated from a dairy source and was identified according to standard taxonomic guidelines. The taxonomy of strain Lb-87 at the genus and species level has been determined by 16S rRNA sequencing and DNA fingerprinting. *L. bulgaricus* Lb-87 belongs to the *L. Lactobacillus delbrueckii* species and *L. Lactobacillus delbrueckii subspecies bulgaricus*. The 500 bp sequence alignment of Lb-87 matched the 16S rRNA region of *L. bulgaricus* with greater than 99% homology. The subspecies was determined by the combined use of the PCR methods described previously (Lick, 2000; Torriani, 1999). According to the taxonomy, *L. bulgaricus* Lb-87 belongs to a species included in the most updated list of Qualified Presumption of Safety (QPS) microorganisms intentionally added to food and feed (EFSA BIOHAZ 2013). DuPont™ Danisco® provides strain Lb-87 as a white to cream-colored freeze-dried probiotic powder for inclusion in food and dietary supplement products globally, and has historically manufactured and commercially offered strains of *L. bulgaricus.*

In vitro, has demonstrated very low gelatinase activity, a specific marker reflecting periodontal destruction, indicating that the species is not harmful for gingival health (Stamatova et al,

2007). In order to assess the safety of L. bulgaricus Lb-87 further, a fourteen-day acute oral toxicity study was conducted in female rats (DuPont, 2015, Internally generated data). At a dose of 5000 mg/kg (=3.62x1011cfu/kg) Lb-87, no mortality, clinical abnormalities, or overall body weight loss was observed. In addition, no gross lesions were present in the rats at necropsy (DuPont, 2015, Internally generated data).

Antibiotic susceptibility patterns Antibiotic susceptibility profiles are an important means of demonstrating the potential of an organism to be readily inactivated by the antibiotics used in human therapy. Antibiotic resistance is a natural property of microorganisms and existed before antibiotics became used by humans. In many cases, resistance is due to the absence of the specific antibiotic target or is a consequence of natural selection. Antibiotic resistance can be defined as the ability of some bacteria to survive or even grow in the presence of certain substances that usually inhibit or kill other bacteria. This resistance may be inherent/intrinsic or acquired.

Inherent or intrinsic resistance Most, if not all, strains of a certain bacterial species are not normally susceptible to a certain antibiotic. The antibiotic has no effect on these cells, being unable to kill or inhibit the bacterium, for example because the target for the antibiotic may be missing. Lb-87 has a single multidrug-efflux transporter gene encoded within its genome, which is hypothesized to provide some level of resistance to fluoroquinolones (Neyfakh et al, 1993; Piddock, 2006)

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